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13. ABSTRACT (Maximum 200 Words)

The goal of our research is to determine if there is a developmental window for suppressing prostate cancer with the phytoestrogen, genistein, and its mechanism (s) of chemoprevention. Genistein in the diet suppressed chemically-induced prostate cancer in rats. Life-time (starting at birth) exposure to genistein was more effective in conferring protection against prostate cancer than neonatal/prepubertal or adult only exposures to genistein, suggesting that developmental and/or programming effects plus maintenance regulation plays a role in protecting against prostate cancer. Neonatal/prepubertal genistein exposure does not alter prostate bud development. Dietary genistein during the neonatal/prepubertal period as well as during adulthood resulted in decreased androgen receptor, but not estrogen receptors alpha and beta, in dorsolateral prostates of 70 day old rats, suggesting that early exposure to genistein can have a programming or imprinting effect on androgen receptor expression. We are in the process of investigating DNA methylation of the androgen receptor promoter as a mechanism of early genistein exposure contributing to prostate cancer chemoprevention.

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Introduction

The disease of cancer is usually attacked at time of diagnosis, and even chemoprevention is not usually considered until adulthood. Our hypothesis is that windows of development hold the key for chemoprevention of prostate cancer. We have previously demonstrated that exposure to physiological concentrations of genistein starting at 5 weeks suppressed the development of chemically-induced prostate cancer and is bioavailable to the prostate in rats. The purpose of our proposed research is to determine if there is a developmental window for this chemoprevention and the mechanism (s) of chemoprevention. The importance of this lies in the need to know, prior to initiation of human trials, if we need to expose infants and/or adults to get maximum chemoprevention.

Body

Aim 1. To determine if a specific window of development (neonatal/prepubertal only, adult only or life-time) is responsible for genistein chemoprevention of prostate cancer. This was carried out in the following groups of rats.
Group A) no genistein in the diet as positive controls.
Group B) genistein in the diet from birth until 35 days of age only.
Group C) genistein in the diet starting at 90 days of age, 20 days after cancer initiation.
Group D) genistein via the diet from birth throughout life to demonstrate that postnatal lifetime exposure only protects against prostate cancer.

Prostate Cancer Incidence in Lobund-Wistar Rats* Fed Genistein in the Diet

		Percent of Rats with Pathological Score:					
Treatment	#/Group	1	2	3	4	5	6
A) Controls	23	22	4	17	4	9	43
B) Genistein (1-35 Days)	27	37	4	15	7	7	30
C) Genistein (3-11 Months)	28	32	25	14	0	0	29[a]
D) Genistein (1-11 Months)	30	43	23	0	3	10	20[a,b]

*Male rats were injected with 42 mg NMU/kg BW into the dorsal prostate on day 70 and necropsied when 11 months old or when moribund. Key to Pathology report: 1 – No tumor; 2 – Low-grade PIN; 3 – High-grade PIN; 4 – Well-differentiated lesion; 5 – Moderately differentiated lesion; 6 – Poorly differentiated lesion. [a] < 0.05 compared to controls based on the exact Cochran-Armitage trend test. [b] < 0.05 compared to Controls only using the chi-square test.

The treatment groups and their tumor grade frequencies were cross-tabulated as a 4 x 6 contingency table (treatment versus tumor grade) and were analyzed using categorical data analysis from the FREQ procedure provided in SAS. An overall likelihood ratio test was used to test for significant association. In addition, each of the treatment groups was compared to the control using the Cochran-Armitage trend test.

From a 4 x 6 contingency table analysis based on the exact likelihood ratio chi-square test, there was significant association between treatments (Controls, Genistein for 1-35 Days, Genistein for 3-11 Months, and Genistein for 1-11 Months) and tumor grade (1-6), p-value = 0.022. However, when comparing each of the treatment groups to the controls, only Genistein for 1-11 months was significantly different than controls (p-value = 0.023). Note that while 43.5% of the Control animals had grade-6 tumors, only 20% of the Genistein for 1-11 months and while only 21.7% of

the Control animals had no tumors (grade-1), 43.3% of the Genistein for 0-11 months had no tumors (grade-1). Genistein for 1-35 days and Genistein for 3-11 months were not significantly different than controls (p-values = 0.887 and 0.072, respectively). In addition, there were no significant differences between Genistein for 1-11 months and Genistein for 1-35 days (p-value=0.075). No other comparisons were significant.

Based on the exact Cochran-Armitage trend test, the Genistein 1-11 month and the Genistein 3-11 month groups had statistically significant downward trends in tumor grade as compared to controls (p-value =0.016, 0.041, respectively). There was no significant trend for Genistein 1-35 days as compared to controls (p-value=0.134). There were no significant differences in trend between any of the Genistein treatment groups.

Aim 2. To investigate prostate gland morphology in the dorsal and lateral lobes of the prostates of 21 and 35 day old rats exposed ± genistein in the diet, starting at birth. No statistical significance was detected for prostate bud perimeter from genistein treatment compared to control treated rats. This work was completed in the first year and was already reported.

Aim 3. To investigate the potential of genistein to regulate sex steroid receptor expression as mechanism of prostate cancer prevention.

Dietary genistein resulted in decreased androgen receptor, but not estrogen receptors alpha and beta, in dorsolateral prostates of 70 day old rats. The finding that neonatal/prepubertal genistein treatment only was able to down-regulate the androgen receptor as well as did adult only and life-time genistein treatments suggested that early exposure to genistein can have a programming or imprinting effect on androgen receptor expression. This is consistent with neonatal/ prepubertal genistein treatment causing permanent effect on gene and protein expression via DNA methylation. This work was completed in the second year and was already reported.

Aim 4. To investigate DNA methylation of AR, ER alpha and ER beta as imprinting mechanism of action.

Based on the data of Aim 3, it is plausible that androgen receptor, but not estrogen receptors alpha and beta, could be regulated via DNA methylation mechanism. Hence, we have attempted to measure DNA methylation via methylation specific PCR (MSP). However, rat androgen receptor MSP primers have not been previously designed. MSP has been commonly implemented to study the methylation status in human cancer samples or human cell lines. Subsequently a large number of MSP primers have been designed to recognize human sequences, but a small number studies have designed primers for rat or mouse promoter sequences. The concept for designing primers and implementing MSP are the same, however we have experienced difficulty in optimizing the PCR conditions. We designed two sets of methylated and unmethylated primers based on the rat androgen receptor promoter region sequence. We used MethPrimer, a program for designing PCR primers for MSP. It takes the promoter region sequence as its input and searches the sequence for potential CpG islands. Primers are then picked around the predicted CpG islands. There was only one 122 bp CpG island identified within the rat androgen receptor promoter region, therefore primer sets were designed around this region (2531-2652). We are currently optimizing the PCR conditions for the above-mentioned primers. Optimization consists of utilizing a MJ Research Gradient Thermal Cycler (PTC-200) to determine the annealing temperature of the primers, varying the concentration of template DNA, and modifying other PCR conditions (i.e. MgCl concentrations,

denature time, extension time, and cycle number). To date, we have not established PCR conditions that produce reliable and reproducible data. The problem may lie in primer design and annealing which is the most difficult step in optimizing PCR conditions. Another potential problem may be incomplete bisulfite conversion of the template DNA. It has been reported that conventional bisulfite treatments can result in a loss of 96% of the starting DNA. To circumvent this, we are purchasing the MethylEasy kit designed by Human Genetic Signatures (Australia) which addresses these shortcomings and performs the DNA modification in one tube requiring no DNA pre-treatment, improves sensitivity, no column purification, and virtually no loss of DNA. Also, if warranted, we plan to develop and evaluate a third primer set if the previous two do not yield satisfactory results. After we have worked out the MSP conditions for the androgen receptor we plan to investigate the degree of methylation using a more sensitive system, ABI 7500 Real-Time PCR. By utilizing Real-time PCR we will be able to quantify the amount of PCR product in the log phase of the reaction instead of after a fixed number of cycles in traditional PCR (end-point analysis), thus allowing a more accurate quantification. Also, melting curve analysis can be used as a confirmative analysis by distinguishing between the melting temperature of methylated and unmethylated PCR products.

Since we have not succeeded in completing this Aim, we are requesting a No Cost Extension to complete the work.

Key Research Accomplishments

- There was significant association between treatments (Controls, Genistein for 1-35 Days, Genistein for 3-11 Months, and Genistein for 1-11 Months) and tumor grade (1-6), p-value = 0.022.

- Based on the exact Cochran-Armitage trend test, the 1-11 month Genistein and the 3-11 month groups Genistein had statistically significant downward trends in tumor grade as compared to controls (p-value =0.016, 0.041, respectively).

- Compared to Controls, only Genistein for 1-11 months was significantly different in tumor grade (p-value = 0.023).

- Neonatal/prepubertal genistein in the diet does not alter prostate bud perimeter in 21 and 35 day old rats. This demonstrates that genistein is not capable of altering rat prostate morphology.

- Dietary genistein during the neonatal/prepubertal period as well as during adulthood resulted in decreased androgen receptor, but not estrogen receptors alpha and beta, in dorsolateral prostates of 70 day old rats, suggesting that early exposure to genistein can have a programming or imprinting effect on androgen receptor expression.

Reportable Outcomes

None.

Conclusion

Genistein in the diet suppressed chemically-induced prostate cancer in rats. Life-time (starting at birth) exposure to genistein is more effective in conferring protection against prostate cancer than neonatal/prepubertal or adult only exposures to genistein, suggesting that developmental and/or programming effects plus maintenance regulation plays a role in protecting against prostate cancer. Neonatal/prepubertal genistein exposure does not alter prostate bud development. Dietary genistein during the neonatal/prepubertal period as well as during adulthood resulted in decreased androgen receptor, but not estrogen receptors alpha and beta, in dorsolateral prostates of 70 day old rats, suggesting that early exposure to genistein can have a programming or imprinting effect on androgen receptor expression.

References

None

Appendices

None